IACUC Guideline
USE OF COMPLETE FREUND’S ADJUVANT
IN LABORATORY ANIMALS

The University of Pennsylvania’s Institutional Animal Care and Use Committee (IACUC) has adopted the following guidelines for the use of Complete Freund’s Adjuvant in laboratory animals. These guidelines are based on the National Institutes of Health Intramural "Guidelines for the Use of Adjuvants in Research" [1]. The IACUC recognizes that the Principal Investigator is best qualified to select the appropriate adjuvant to be used, and the committee will consider justified applications for use of adjuvants outside these guidelines.

Use of Complete Freund’s Adjuvant (CFA) must be specifically addressed in the protocol application, scientifically justified, and a comprehensive search for alternatives considered.

These Guidelines are specific to the use of Complete Freund’s Adjuvant; additional general information or information on the use of any other adjuvants may be found in the UPenn IACUC Guideline “Monoclonal and Polyclonal Antibody Production.”

Background

CFA is a water-in-oil emulsion containing killed, dried Mycobacterium butyricum which has been used to enhance antigenicity and stimulate an immune response greater than antigen alone. Incomplete Freund's Adjuvant (IFA), water-in-oil emulsion only, is used for similar reasons. The intention of the following guidelines is to minimize potential animal discomfort associated with the use of CFA in research.

Reduction, Replacement and Refinement

The USDA has determined that the use of CFA may cause more than momentary or slight pain and may cause a severe inflammatory reaction, depending on the species and route of administration [4]. The improper or unnecessary use of CFA may cause severe inflammation, indurations, and/or necrosis in laboratory animals [2]. The most severe inflammatory responses in animals are seen following multiple injections of CFA.

Non-painful alternatives must be considered and documented as part of a written narrative describing a literature search for alternatives to the use of CFA [5]. For more information pertaining to adequate literature search techniques, see UPenn IACUC guideline Literature Search for Alternatives. Alternatives to consider include those which reduce the number of animals required (e.g. tissue culture, chicken eggs) or utilize less traumatic adjuvants. Potential adjuvants or antibody production alternatives may specifically include:

- Incomplete Freund’s Adjuvant (IFA)
- RIBI®
- TiterMax®
- Specol®
- Montamides
- Syntex Adjuvant Formation (SAF)
- Aluminum compounds

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- Subcutaneously implanted chambers
- SuperCarrier®
- Elvax®
- L-tyrosine
- AdjuPrime®
- Nitrocellulose-absorbed protein
- Gerbu adjuvant
- Immune-stimulating complexes (ISCOMS)

Guidelines

1. Non-inflammatory adjuvants or adjuvants that produce a less intense inflammatory response should be strongly considered as an alternative to CFA [3]. CFA must be used only when absolutely necessary and its use must be justified in each protocol.
2. If CFA is to be used, it must be limited to the initial immunizing dose. Any subsequent immunizations should use an alternative adjuvant such as IFA. If more than one dose of CFA is proposed, it must be strongly justified with objective data.
3. NIH Guidelines must be followed, regardless of the route of CFA administration (injection site, dose, or volume) [1]. Typical routes of injection are listed below (Table 1). The IACUC recommends the use of subcutaneous injections in all species. Intraperitoneal (IP) and intramuscular (IM) injections in mice, rats and other small rodents should be avoided.

**Intravenous (IV) administration is prohibited.**

- If intramuscular injections must be performed, fewer complications occur following injection in the lumbar muscles compared to the hindlimbs. Therefore, lumbar administration is acceptable.
- Intradermal injection, in particular, may result in skin necrosis and sloughing. Scientific justification for this route of injection must be provided
- Avoid sites of injection which are weight bearing (e.g., footpad), used in restraint, or prone to self-mutilation.
- If footpad injection is scientifically justified, then only one footpad may be injected in each experimental animal.

<table>
<thead>
<tr>
<th>Table 1: Recommended Volume of CFA-Antigen Emulsion (CFA-AE) per Site and Route of Administration</th>
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<tbody>
<tr>
<td><strong>Species</strong></td>
</tr>
<tr>
<td>Mouse</td>
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<td>Rat</td>
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<tr>
<td>Rabbit</td>
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<td>Goat/Sheep</td>
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* Not recommended
** Only When Justified
*** Only One Limb Recommended Without Justification
NA: Not applicable
4. Injection sites should be clipped free of hair and surgically scrubbed. The use of sterile needles and syringes is mandatory to minimize microbial contamination. Glass syringes may be preferable as the rubber plungers of certain plastic syringes may react with the oil in the adjuvant.

5. The CFA:antigen emulsified mixture of 1:1 is commonly used. The inoculum must be sterile. The antigen should be microfiltered (e.g. Millipore-filtered) to be free of extraneous microbial or other particulate contamination prior to mixing with the adjuvant. If the emulsion is properly prepared [6], a 23-gauge needle on a 0.5 ml syringe will allow for accurate dosing.

6. Injection sites must be separated from each other widely enough to ensure continued blood supply to adjacent areas of skin and subcutaneous tissues.

7. To reduce excessive inflammatory response, preparations of CFA with a lower mycobacterial concentration (i.e., 0.05 mg/ml to a maximum of 0.5 mg/ml) should be chosen [7].

Post-injection Monitoring
Post-injection observations should be performed twice-weekly for up to four weeks. If pain, distress, or complications at the inoculation site occur (e.g. ulceration), the animal must be reported as a “sick animal” and ULAR veterinary services must be contacted and a treatment plan created for the animal.

References

1. ARAC. Guidelines for the Use of Adjuvants in Research. NIH Intramural Guidelines. 2010.


